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     (FILE 'HOME' ENTERED AT 11:37:27 ON 11 SEP 2003)

     FILE 'BIOSIS, CAPLUS, EMBASE, MEDLINE, CANCERLIT, JAPIO' ENTERED AT
     11:41:55 ON 11 SEP 2003
L1           163 S (ANTIBOD? ARRAY)
L2            53 S L1 AND BIND?
L3            10 S L2 AND PATTERN?
L4             5 S L3 AND CELL
L5             1 S L4 AND LYSATE?
L6             5 DUPLICATE REMOVE L4 (0 DUPLICATES REMOVED)
L7             0 S L4 NOT L3
L8             5 S L3 NOT L4
L9             5 DUPLICATE REMOVE L8 (0 DUPLICATES REMOVED)

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L9 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1997:424428 CAPLUS
DN 127:170868
TI Affinity characterization of monoclonal and recombinant antibodies for multianalyte detection with an optical transducer
AU Piehler, Jacob; Brecht, Andreas; Giersch, Thomas; Kramer, Karl; Hock, Bertold; Gauglitz, Guenter
CS Universitaet Tuebingen, Institut fuer Physikalische und Theoretische Chemie, Auf der Morgenstelle 8, D-72076, Tubingen, Germany
SO Sensors and Actuators, B: Chemical (1997), B39(1-3), 432-437
CODEN: SABCEB; ISSN: 0925-4005
PB Elsevier
DT Journal
LA English
CC 80-6 (Organic Analytical Chemistry)
Section cross-reference(s): 5, 9, 28
AB The selectivity of immunoassay is limited by the cross-reactivity of antibodies to structurally related analytes. This becomes a drawback for applications that require discrimination of slightly different analytes. An approach to overcoming this problem is the application of **antibody arrays** that show differences in their affinity **patterns**. The authors have studied this method using systematic modeling of multianalyte systems based on test-independent affinity parameters. A model system of anti-s-triazine antibodies and s-triazine derivs. was studied. The immunoassay is carried out in an indirect test format using an optical transducer for label-free monitoring of antibody **binding** at an immobilized hapten. The concn. of free antibody in equil. with the analyte is probed in a flow-through system. This format allows simple modeling of the response and assessment of the affinity const. from the calibration curve. The affinity **patterns** of five monoclonal antibodies and a recombinant single-chain fragment with respect to five s-triazine derivs. are detd. by this method. An array of three antibodies is selected and the response **pattern** to mixts. of three analytes detd. Measured and calcd. **pattern** correspond in principle, but systematic deviations are obsd. due to the perturbation of equil. during detection. The correlation of the true analyte concn. and the analyte concns. predicted from the signal **pattern** using the affinity consts. strongly depend on the selectivity and the affinity of the antibodies.
ST monoclonal recombinant antibody multianalyte detn; triazine herbicide detn immunoassay **antibody array**; antibody **binding** triazine reflectometric interference spectroscopy
IT Antibodies
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(monoclonal; triazine herbicides detn. by immunoassay with **antibody arrays** and reflectometric interference spectroscopy for label-free monitoring of antibody **binding** at immobilized hapten)
IT Immunoassay
Pattern recognition
(triazine herbicides detn. by immunoassay with **antibody arrays** and reflectometric interference spectroscopy for label-free monitoring of antibody **binding** at immobilized hapten)
IT Herbicides
(triazine; triazine herbicides detn. by immunoassay with **antibody arrays** and reflectometric interference spectroscopy for label-free monitoring of antibody **binding** at immobilized hapten)
IT 122-34-9, Simazine 139-40-2, Propazine 1912-24-9, Atrazine 5915-41-3, Terbuthylazine 6190-65-4, De-ethylatrazine
RL: ANT (Analyte); ANST (Analytical study)
(triazine herbicides detn. by immunoassay with **antibody arrays** and reflectometric interference spectroscopy for

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IT Antibodies
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(monoclonal; triazine herbicides detn. by immunoassay with **antibody arrays** and reflectometric interference spectroscopy for label-free monitoring of antibody **binding** at immobilized hapten)
IT Immunoassay
Pattern recognition
(triazine herbicides detn. by immunoassay with **antibody arrays** and reflectometric interference spectroscopy for label-free monitoring of antibody **binding** at immobilized hapten)
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RL: ANT (Analyte); ANST (Analytical study)
(triazine herbicides detn. by immunoassay with **antibody arrays** and reflectometric interference spectroscopy for

label-free monitoring of antibody **binding** at immobilized hapten)

label-free monitoring of antibody **binding** at immobilized hapten)

9 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1996:53591 CAPLUS
DN 124:169871
TI Multi-analyte determination with a direct optical multi-antibody detection system
AU Piehler, Jacob; Brecht, Andreas; Kramer, Karl; Hock, Bertold; Gauglitz, Guenter
CS Institut fur Physikalische und Theoretische Chemie, Universitat Tubingen, Tuebingen, D-72076, Germany
SO Proceedings of SPIE-The International Society for Optical Engineering (1995), 2504(Environmental Monitoring and Hazardous Waste Site Remediation, 1995), 185-94
CODEN: PSISDG; ISSN: 0277-786X
PB SPIE-The International Society for Optical Engineering
DT Journal
LA English
CC 9-10 (Biochemical Methods)
AB Discrimination of structurally similar analytes by immunoassay is limited by antibody cross reactivity. Using a plurality of cross-reacting antibody species allows increased selectivity by application of **pattern** recognition methods. We present a detailed characterization of an array of monoclonal antibodies which allows anal. modeling of the performance of an **antibody array** in a multi-analyte system. Such well defined **antibody arrays** give the possibility for the systematical optimization for immunoassay applications. Affinity characterization is carried out in a simple test format: After equil. **binding** of antibody and analyte, unoccupied antibody is quantified by an optical transducer. The test result reflects directly the resp. affinity consts. for different analytes. A set of three monoclonal antibodies was characterized with respect to their affinity to five different triazines which play an important role in water contamination. The affinities were compared with results obtained by direct enzyme immunoassay. The anal. performance of the **antibody array** was modelled by using the affinity consts. detd. from the calibration curve.
ST antibody immunoassay triazine detn
IT Immunoassay
(multi-analyte detn. with a direct optical multi-antibody detection system)
IT Antibodies
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(multi-analyte detn. with a direct optical multi-antibody detection system)
IT 122-34-9, Simazine 139-40-2, Propazine 1912-24-9 5915-41-3, Terbutylazine 6190-65-4, De-ethylatrazine
RL: ANT (Analyte); ANST (Analytical study)
(multi-analyte detn. with a direct optical multi-antibody detection system)

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ST antibody immunoassay triazine detn
IT Immunoassay
(multi-analyte detn. with a direct optical multi-antibody detection system)
IT Antibodies
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(multi-analyte detn. with a direct optical multi-antibody detection system)
IT 122-34-9, Simazine 139-40-2, Propazine 1912-24-9 5915-41-3, Terbutylazine 6190-65-4, De-ethylatrazine
RL: ANT (Analyte); ANST (Analytical study)
(multi-analyte detn. with a direct optical multi-antibody detection system)

L5 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2003 ACS on STN

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AN 1994:574362 CAPLUS
DN 121:174362
TI Matrix-Assisted Laser Desorption/Ionization Using an Active Perfluorosulfonated Ionomer Film Substrate
AU Bai, Jian; Liu, Yan-Hui; Cain, Teresa C.; Lubman, David M.
CS Department of Chemistry, University of Michigan, Ann Arbor, MI, 48109-1055, USA
SO Analytical Chemistry (1994), 66(20), 3423-30
CODEN: ANCHAM; ISSN: 0003-2700
DT Journal
LA English
CC 9-5 (Biochemical Methods)
Section cross-reference(s): 6, 73
AB The use of an active Nafion substrate is shown to enhance the performance of MALDI MS. The use of a Nafion substrate with certain matrixes can significantly enhance the signals obtained over those obsd. with a stainless steel probe substrate. Analytes can often be obsd. with the use of the Nafion substrate that cannot be easily obsd. with the std. MALDI procedure, and usually a much wider range of peaks can be obsd. using MALDI from the Nafion substrate than with any single matrix on a stainless steel substrate. This enhancement of signal from the Nafion substrate is obsd. only with the sequential deposition of the sample and the matrix onto the Nafion film. If the analyte and matrix are premixed, then the effect is not obsd. The use of the Nafion substrate has been shown to be particularly effective in analyzing real biol. mixts. without prepurifn. This has been demonstrated for various samples including the anal. of the products of chem. digests of proteins, **protein profiling** in milk and egg white samples, **cell lysate** anal., and oligonucleotide detection.
ST matrix assisted mass spectrometry Nafion substrate; biopolymer mass spectrometry Nafion substrate
IT Protein hydrolyzates
RL: ANT (Analyte); ANST (Analytical study)
(anal. of, by matrix-assisted laser desorption/ionization mass spectrometry with Nafion film substrate)
IT Biopolymers
Proteins, properties
RL: PRP (Properties)
(mass spectrometry of, matrix-assisted laser desorption/ionization, Nafion film substrate in)
IT Nucleotides, analysis
RL: ANT (Analyte); ANST (Analytical study)
(oligo-, detection of, by matrix-assisted laser desorption/ionization mass spectrometry with Nafion film substrate)
IT Mass spectrometry
(photodesorption/photoionization, laser-induced, matrix-assisted, Nafion substrate in)
IT Ionomers
RL: ANST (Analytical study)
(polyoxyalkylenes, fluorine- and sulfo-contg., film, as substrate in matrix-assisted laser desorption/ionization mass spectrometry)

L5 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1994:574362 CAPLUS
DN 121:174362
TI Matrix-Assisted Laser Desorption/Ionization Using an Active Perfluorosulfonated Ionomer Film Substrate
AU Bai, Jian; Liu, Yan-Hui; Cain, Teresa C.; Lubman, David M.
CS Department of Chemistry, University of Michigan, Ann Arbor, MI, 48109-1055, USA
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Section cross-reference(s): 6, 73
AB The use of an active Nafion substrate is shown to enhance the performance of MALDI MS. The use of a Nafion substrate with certain matrixes can significantly enhance the signals obtained over those obsd. with a stainless steel probe substrate. Analytes can often be obsd. with the use of the Nafion substrate that cannot be easily obsd. with the std. MALDI procedure, and usually a much wider range of peaks can be obsd. using MALDI from the Nafion substrate than with any single matrix on a stainless steel substrate. This enhancement of signal from the Nafion substrate is obsd. only with the sequential deposition of the sample and the matrix onto the Nafion film. If the analyte and matrix are premixed, then the effect is not obsd. The use of the Nafion substrate has been shown to be particularly effective in analyzing real biol. mixts. without prepurifn. This has been demonstrated for various samples including the anal. of the products of chem. digests of proteins, **protein profiling** in milk and egg white samples, **cell lysate** anal., and oligonucleotide detection.
ST matrix assisted mass spectrometry Nafion substrate; biopolymer mass spectrometry Nafion substrate
IT Protein hydrolyzates
RL: ANT (Analyte); ANST (Analytical study)
(anal. of, by matrix-assisted laser desorption/ionization mass spectrometry with Nafion film substrate)
IT Biopolymers
Proteins, properties
RL: PRP (Properties)
(mass spectrometry of, matrix-assisted laser desorption/ionization, Nafion film substrate in)
IT Nucleotides, analysis
RL: ANT (Analyte); ANST (Analytical study)
(oligo-, detection of, by matrix-assisted laser desorption/ionization mass spectrometry with Nafion film substrate)
IT Mass spectrometry
(photodesorption/photoionization, laser-induced, matrix-assisted, Nafion substrate in)
IT Ionomers
RL: ANST (Analytical study)
(polyoxyalkylenes, fluorine- and sulfo-contg., film, as substrate in matrix-assisted laser desorption/ionization mass spectrometry)

WEST

End of Result Set

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L15: Entry 12 of 12 File: USPT May 22, 1990

DOCUMENT-IDENTIFIER: US 4927502 A
TITLE: Methods and apparatus using galvanic immunoelectrodes

Other Reference Publication (2):
G. Robinson et al., "Bioelectrochemical Immunoassay for Human Chorionic Gonadotrophin in Serum Using an Electrode--Immobilized Capture Antibody", Biosensors, vol. 2, at 45-57 (1986).

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L6: Entry 47 of 87 File: USPT Sep 17, 2002

DOCUMENT-IDENTIFIER: US 6453241 B1
TITLE: Method and system for analyzing biological response signal data

Drawing Description Text (22):
FIG. 23 shows a "resolved" profile that is the result of subtracting an Experiment profile of yeast cells harboring an impaired version of a gene, ERG11 and an Experiment profile of yeast cells treated with the drug clotrimazole.

Detailed Description Text (5):
Preferred embodiments are described herein with respect to one example of a system yielding biological response signals, although it is to be appreciated that the scope of the preferred embodiments is not so limited and may be applied to any of a variety of experimental environments involving biological response signals. An expression array is a microarray adapted to generate light signals at each matrix site responsive to an amount of mRNA being expressed for a particular gene product at that site. Such systems are generally described in U.S. Pat. No. 6,203,987, "Methods for Using Co-regulated Genesets to Enhance Detection and Classification of Gene Expression Patterns," filed Oct. 27, 1998; U.S. patent application Ser. No. 09/220,142, "Methods of Characterizing Drug Activities Using Consensus Profiles," filed Dec. 23, 1998; U.S. patent application Ser. No. 09/220,274, "Methods for Robust Discrimination of Profiles," filed Dec. 23, 1998; and U.S. patent application Ser. No. 09/220,275, "Methods for Using Co-regulated Genesets to Enhance Detection and Classification of Gene Expression Patterns,". The contents of each of the these applications is hereby incorporated by reference into the present application. Moreover, all publications cited herein are incorporated by reference in their entirety.

Detailed Description Text (69):
FIG. 16 shows steps for computer-assisted analysis of biological response data in accordance with a preferred embodiment, the steps being used in conjunction with the biological response analysis software 304. It has been found that it is desirable not only to provide computer software for analyzing biological data using the separated biological viewers disclosed supra, but also to integrate these biological viewers by allowing the projection of selected datasets onto these biological viewers. In one preferred embodiment, the dataset to be projected is selected according to search methods disclosed supra and projected onto all currently active biological viewers, using appropriate highlighting such as color highlighting. In another preferred embodiment, a source dataset is selected from a source viewer, i.e., a first currently active biological viewer, and projected onto one or more destination viewers, i.e., one or more of the other currently active biological viewers. The source dataset is projected onto the destination biological viewers through the highlighting of destination data points that correspond to the source dataset, making these data points stand out for clear recognition by the user, thus enhancing the ability to recognize relationships, trends, patterns, etc. in the biological response database. This also allows the user to identify a meaningful response using a viewer well-suited to a particular purpose, then see the same response in other viewers to support or refute the discovery.

Detailed Description Text (84):
The approach illustrated in this section has been used to identify experiments in which a particular gene or collection of genes is perturbed or affected. In the example of FIG. 5, a gene signal profile for a yeast gene RNR2 has been constructed using the RNR2 component of 863 biological signal profiles for experiments involving yeast gene expression monitoring of samples derived from cells with a variety of drug

treatments or genetic perturbations. The profile exposes that RNR2 transcript levels are elevated with increasing concentration of the drug methotrexate (labeled MTX on plot). In that example, all experiments chosen to construct the RNR2 signal profile have a common baseline: yeast "wild type" untreated cells.

Detailed Description Text (85):
With reference to FIG. 22 and 23, in accordance with the preferred embodiments, a "Resolve" feature is provided that allows subtraction of biological signal profiles or combinations thereof from one another. The resolve feature can be used in many circumstances to validate drug targets. As shown in FIG. 22 and FIG. 23, exemplary profiles such as, an Experiment profile based on yeast cells harboring an impaired version of a gene, ERG11, which is the target of the antifungal drug clotrimazole (ERG11; at left on FIG. 23), along with an Experiment profile of yeast cells treated with the drug clotrimazole (tet; at right on FIG. 23) can serve as input to the "Resolve" feature. The "resolved" profile, showing the differences between the two profiles (clotrimazole treated cell profile minus tet-ERG11 cell profile) is shown at the bottom of FIG. 23. The only significant outlier is the ERG11 gene, because it was turned off in the tet-ERG11 experiment, but remains active in the drug treated cell. This is indicative of a very precise drug that closely mimics the cellular effects of the deletion of its target. As this example illustrates, the "resolve" feature represents an method for validating the effects of drugs or for validating drug targets. While this illustration demonstrates the subtraction of one Experiment profile from an other, one skilled in the art will appreciate that combinations of profiles may be subtracted from a single profile or from yet other combinations of profiles.

Detailed Description Text (92):
To measure drug response data, cell are exposed to graded levels of the drug or drug candidate of interest. When the cells are grown in vitro, the compound is usually added to their nutrient medium. In the case of yeast, such as S. cerevisiae, it is preferably to harvest the cells in early log phase, since expression patterns are relatively insensitive to time of harvest at that time. The drug is added in a graded amount that depends on the particular characteristics of the drug, but usually will be between about 1 ng/ml and 100 mg/ml. In some cases a drug will be solubilized in a solvent such as DMSO.

Detailed Description Text (93):
The biological state of cells exposed to the drug and cells not exposed to the drug is measured according to any of the below described methods. Preferably, transcript or microarrays are used to find the mRNAs with altered expression due to exposure to the drug. However, other aspects of the biological state may also be measured to determine, e.g., proteins with altered translation or activities due to exposure to the drug.

Detailed Description Text (96):
In general, measurement of the transcriptional state can be performed using any probe or probes which comprise a polynucleotide sequence and which are immobilized to a solid support or surface. For example, the probes may comprise DNA sequences, RNA sequences, or copolymer sequences of DNA and RNA. The polynucleotide sequences of the probes may also comprise DNA and/or RNA analogues, or combinations thereof. For example, the polynucleotide sequences of the probe may be full or partial sequences of genomic DNA, cDNA, or mRNA sequences extracted from cells. The polynucleotide sequences of the probes may also be synthesized nucleotide sequences, such as synthetic oligonucleotide sequences. The probe sequences can be synthesized either enzymatically in vivo, enzymatically in vitro, (e.g., by PCR), or non-enzymatically in vitro.

Detailed Description Text (99):
In a particularly preferred embodiment, measurement of the transcriptional state are made by hybridization to microarrays of probes consisting of a solid phase, on the surface of which are immobilized a population of polynucleotides, such as a population of DNA or DNA mimics, or, alternatively, a population of RNA. Specifically, a microarray is an array of less than 6.25 cm.sup.2 in size. Microarrays can be employed, e.g., for analyzing the transcriptional state of a cell, such as the transcriptional states of cells exposed to graded levels of a drug of interest.

Detailed Description Text (114):
As described, supra, the polynucleotide molecules which may be analyzed by the present invention may be from any source, including naturally occurring nucleic acid molecules, as well as synthetic nucleic acid molecules. In a preferred embodiment, the polynucleotide molecules analyzed by the invention comprise RNA, including, but by no means limited to, total cellular RNA, poly(A).sup.- messenger RNA (mRNA), fractions thereof, or RNA transcribed from cDNA. Methods for preparing total and poly(A).sup.+ RNA are well known in the art, and are described generally, e.g., in Sambrook et al., supra. In one embodiment, RNA is extracted from cells of the various types of interest in this invention using guanidinium thiocyanate lysis followed by CsCl centrifugation (Chirgwin et al., 1979, Biochemistry 18:5294-5299). Poly (A).sup.+ RNA is selected by selection with oligo-dT cellulose. Cells of interest include, but are by no means limited to, wild-type cells, drug-exposed wild-type cells, modified cells, diseased cells, and, in particular, cancer cells.

Detailed Description Text (123):
In preferred embodiments, cDNAs from two different cells are hybridized to the binding sites of the microarray. In the case of drug responses, one cell is exposed to a drug and another cell of the same type is not exposed to the drug. The cDNA derived from each of the two cell types are differently labeled so that they can be distinguished. In one embodiment, for example, cDNA from a cell treated with a drug is synthesized using a fluorescein-labeled dNTP, and cDNA from a second cell, not drug-exposed, is synthesized using a rhodamine-labeled DNTP. When the two cDNAs are mixed and hybridized to the microarray, the relative intensity of signal from each cDNA set is determined for each site on the array, and any relative difference in abundance of a particular mRNA is thereby detected.

Detailed Description Text (124):
In the example described above, the cDNA from the drug-treated cell will fluoresce green when the fluorophore is stimulated, and the cDNA from the untreated cell will fluoresce red. As a result, when the drug treatment has no effect, either directly or indirectly, on the relative abundance of a particular mRNA in a cell, the mRNA will be equally prevalent in both cells, and, upon reverse transcription, red-labeled and green-labeled cDNA will be equally prevalent. When hybridized to the microarray, the binding site(s) for that species of RNA will emit wavelength characteristic of both fluorophores. In contrast, when the drug-exposed cell is treated with a drug that, directly or indirectly, increases the prevalence of the mRNA in the cell, the ratio of green to red fluorescence will increase. When the drug decreases the mRNA prevalence, the ratio will decrease.

Detailed Description Text (128):
According to the method of the invention, the relative abundance of an mRNA in two cells or cell lines is scored as a perturbation and its magnitude determined (i.e., the abundance is different in the two sources of mRNA tested) or as not perturbed (i.e., the relative abundance is the same). As used herein, a difference between the two sources of RNA of at least a factor of about 25% (i.e., RNA is 25% more abundant in one source than in the other source), more usually about 50%, even more often by a factor of about 2 (i.e., twice as abundant), 3 (three times as abundant), or 5 (five times as abundant) is scored as a perturbation. Present detection methods allow reliable detection of difference of an order of about 3-fold to about 5-fold, but more sensitive methods are expected to be developed.

Detailed Description Text (137):
Measurements of the translational state may be performed according to several methods. For example, whole genome monitoring of protein (i.e., the "proteome," Goffea et al., supra) can be carried out by constructing a microarray in which binding sites comprise immobilized, preferably monoclonal, antibodies specific to a plurality of protein species encoded by the cell genome. Preferably, antibodies are present for a substantial fraction of the encoded proteins, or at least for those proteins relevant to the action of a drug of interest. Methods for making monoclonal antibodies are well known (see, e.g., Harlow and Lane, 1988, Antibodies: A Laboratory Manual, Cold Spring Harbor, N.Y.). In a preferred embodiment, monoclonal antibodies are raised against synthetic peptide fragments designed based on genomic sequence of the cell. With such an antibody array, proteins from the cell are contacted to the array, and their

binding is assayed with assays known in the art.

Detailed Description Text (138):
Alternatively, proteins can be separated by two-dimensional gel electrophoresis systems. Two-dimensional gel electrophoresis is well known in the art, and typically involves iso-electric focusing along a first dimension followed by SDS-PAGE electrophoresis along a second dimension. See, e.g., Hames et al., 1990, Gel Electrophoresis of Proteins: A Practical Approach, IRL Press, New York; Shevchenko et al., 1996, Proc. Natl. Acad. Sci. U.S.A. 93:1440-1445; Sagliocco et al., 1996, Yeast 12:1519-1533; and Lander, 1996, Science 274:536-539. The resulting electropherograms can be analyzed by numerous techniques, including mass spectrometric techniques, western blotting, and immunoblot analysis using polyclonal and monoclonal antibodies, and internal and N-terminal micro-sequencing. Using these techniques, it is possible to identify a substantial fraction of all the proteins produced under given physiological conditions, including in cells (e.g., in yeast) exposed to a drug, or in cells modified by, e.g., deletion or over-expression of a specific gene.

Detailed Description Text (146):
Pathway perturbations are preferably made in cells of cell types derived from any organism for which genomic or expressed sequence information is available and for which methods are available that permit controllably modification of the expression of specific genes. Genome sequencing is currently underway for several eukaryotic organisms, including humans, nematodes, Arabidopsis, and flies. In a preferred embodiment, the invention is carried out using a yeast, with Saccharomyces cerevisiae most preferred because the sequence of the entire genome of a S. cerevisiae strain has been determined. In addition, well-established methods are available for controllably modifying expression of year genes. A preferred strain of yeast is a S. cerevisiae strain for which yeast genomic sequence is known, such as strain S288C or substantially isogeneic derivatives of it (see, e.g., Dujon et al., 1994, Nature 369:371-378; Bussey et al., 1995, Proc. Natl. Acad. Sci. U.S.A. 92:3809-3813; Feldmann et al., 1994, E.M.B.O. J 13:5795-5809; Johnston et al., 1994, Science 265:2077-2082; Galibert et al., 1996, E.M.B.O. J. 15:2031-2049). However, other strains may be used as well. Yeast strains are available, e.g., from American Type Culture Collection, 10801 University Boulevard, Manassas, Va, 20110-2209. Standard techniques for manipulating yeast are described in C. Kaiser, S. Michaelis, & A. Mitchell, 1994, Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual, Cold Spring Harbor Laboratory Press, New York; and Sherman et al., 1986, Methods in Yeast Genetics: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor. N.Y.

Detailed Description Text (151):
One disadvantage of the above listed expression systems is that control of promoter activity (effected by, e.g., changes in carbon source, removal of certain amino acids), often causes other changes in cellular physiology which independently alter the expression levels of other genes. A recently developed system for yeast, the Tet system, alleviates this problem to a large extent (Gari et al., 1997, Yeast 13:837-848). The Tet promoter, adopted from mammalian expression systems (Gossen et al., 1995, Proc. Nat. Acad. Sci. USA 89:5547-5551) is modulated by the concentration of the antibiotic tetracycline or the structurally related compound doxycycline. Thus, in the absence of doxycycline, the promoter induces a high level of expression, and the addition of increasing levels of doxycycline causes increased repression of promoter activity. Intermediate levels gene expression can be achieved in the steady state by addition of intermediate levels of drug. Furthermore, levels of doxycycline that give maximal repression of promoter activity (10 micrograms/ml) have no significant effect on the growth rate on wild type yeast cells (Gari et al., 1997, Yeast 13:837-848).

Detailed Description Text (152):
In mammalian cells, several means of titrating expression of genes are available (Spencer, 1996, Trends Genet. 12:181-187). As mentioned above, the Tet system is widely used, both in its original form, the "forward" system, in which addition of doxycycline represses transcription, and in the newer "reverse" system, in which doxycycline addition stimulates transcription (Gossen et al., 1995, Proc. Natl. Acad. Sci. USA 89:5547-5551; Hoffmann et al., 1997, Nucl. Acids. Res. 25:1078-1079; Hofmann et al., 1996, Proc. Natl. Acad. Sci. USA 83:5185-5190; Paulus et al., 1996, Journal of

Virology 70:62-67). Another commonly used controllable promoter system in mammalian cells is the ecdysone-inducible system developed by Evans and colleagues (No et al., 1996, Proc. Nat. Acad. Sci. USA 93:3346-3351), where expression is controlled by the level of muristerone added to the cultured cells. Finally, expression can be modulated using the "chemical-induced dimerization" (CID) system developed by Schreiber, Crabtree, and colleagues (Belshaw et al., 1996, Proc. Nat. Acad. Sci. USA 93:4604-4607; Spencer, 1996, Trends Genet. 12:181-187) and similar systems in yeast. In this system, the gene of interest is put under the control of the CID-responsive promoter, and transfected into cells expressing two different hybrid proteins, one comprised of a DNA-binding domain fused to FKBP12, which binds FK506. The other hybrid protein contains a transcriptional activation domain also fused to FKBP12. The CID inducing molecule is FK1012, a homodimeric version of FK506 that is able to bind simultaneously both the DNA binding and transcriptional activating hybrid proteins. In the graded presence of FK1012, graded transcription of the controlled gene is activated.

Detailed Description Text (153):
For each of the mammalian expression systems described above, as is widely known to those of skill in the art, the gene of interest is put under the control of the controllable promoter, and a plasmid harboring this construct along with an antibiotic resistance gene is transfected into cultured mammalian cells. In general, the plasmid DNA integrates into the genome, and drug resistant colonies are selected and screened for appropriate expression of the regulated gene. Alternatively, the regulated gene can be inserted into an episomal plasmid such as pCEP4 (Invitrogen, Inc.), which contains components of the Epstein-Barr virus necessary for plasmid replication.

Detailed Description Text (154):
In a preferred embodiment, titratable expression systems, such as the ones described above, are introduced for use into cells or organisms lacking the corresponding endogenous gene and/or gene activity, e.g., organisms in which the endogenous gene has been disrupted or deleted. Methods for producing such "knock outs" are well known to those of skill in the art, see e.g., Pettitt et al., 1996, Development 122:4149-4157; Spradling et al., 1995, Proc. Natl. Acad. Sci. USA, 92:10824-10830; Ramirez-Solis et al., 1993, Methods Enzymol. 225:855-878; and Thomas et al., 1987, Cell 51:503-512.

Detailed Description Text (155):
5.3.2. Transfection Systems for Mammalian Cells

Detailed Description Text (156):
Transfection or viral transduction of target genes can introduce controllable perturbations in biological pathways in mammalian cells. Preferably, transfection or transduction of a target gene can be used with cells that do not naturally express the target gene of interest. Such non-expressing, cells can be derived from a tissue not normally expressing the target gene or the target gene can be specifically mutated in the cell. The target gene of interest can be cloned into one of many mammalian expression plasmids, for example, the pcDNA3.1 +/- system (Invitrogen, Inc.) or retroviral vectors, and introduced into the non-expressing host cells. Transfected or transduced cells expressing the target gene may be isolated by selection for a drug resistance marker encoded by the expression vector. The level of gene transcription is monotonically related to the transfection dosage. In this way, the effects of varying levels of the target gene may be investigated.

Detailed Description Text (157):
A particular example of the use of this method is the search for drugs that target the src-family protein tyrosine kinase, lck, a key component of the T cell receptor activation pathway (Anderson et al., 1994, Adv. Immunol. 56:171-178). Inhibitors of this enzyme are of interest as potential immunosuppressive drugs (Hanke J H, 1996, J. Biol Chem 271(2):695-701). A specific mutant of the Jurkat T cell line (JcaM1) is available that does not express lck kinase (Straus et al., 1992, Cell 70:585-593). Therefore, introduction of the lck gene into JCaM1 by transfection or transduction permits specific perturbation of pathways of T cell activation regulated by the lck kinase. The efficiency of transfection or transduction, and thus the level of perturbation, is dose related. The method is generally useful for providing perturbations of gene expression or protein abundances in cells not normally expressing the genes to be perturbed.

Detailed Description Text (161):
Ribozymes can be routinely expressed in vivo in sufficient number to be catalytically effective in cleaving mRNA, and then by modifying mRNA abundances in a cell. (Cotten et al., 1989, EMBO J. 8:3861-3866). In particular, a ribozyme coding DNA sequence, designed according to the previous rules and synthesized, for example, by standard phosphoramidite chemistry, can be ligated into a restriction enzyme site in the anticodon stem and loop of a gene encoding a tRNA, which can then be transformed into and expressed in a cell of interest by methods routine in the art. Preferably, an inducible promoter (e.g., a glucocorticoid or a tetracycline response element) is also introduced into this construct so that ribozyme expression can be selectively controlled. tDNA genes (i.e., genes encoding tRNAs) are useful in this application because of their small size, high rate of transcription, and ubiquitous expression in different kinds of tissues. Therefore, ribozymes can be routinely designed to cleave virtually any mRNA sequence, and a cell can be routinely transformed with DNA coding for such ribozyme sequences such that a controllable and catalytically effective amount of the ribozyme is expressed. Accordingly the abundance of virtually any RNA species in a cell can be perturbed.

Detailed Description Text (173):
In an alternative embodiment, the antisense nucleic acids of the invention are controllably expressed intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced in vivo such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequences encoding the antisense RNAs can be by any promoter known in the art to act in a cell of interest. Such promoters can be inducible or constitutive. Most preferably, promoters are controllable or inducible by the administration of an exogenous moiety in order to achieve controlled expression of the antisense oligonucleotide. Such controllable promoters include the Tet promoter. Less preferably usable promoters for mammalian cells include, but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290: 304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, Cell 22: 787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. USA. 78: 1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296: 39-42), etc.

Detailed Description Text (179):
Antibodies can be introduced into cells in numerous fashions, including, for example, microinjection of antibodies into a cell (Morgan et al., 1988, Immunology Today 9:84-86) or transforming hybridoma mRNA encoding a desired antibody into a cell (Burke et al., 1984, Cell 36:847-858). In a further technique, recombinant antibodies can be engineering and ectopically expressed in a wide variety of non-lymphoid cell types to bind to target proteins as well as to block target protein activities (Biocca et al., 1995, Trends in Cell Biology 5:248-252). Preferably, expression of the antibody is under control of a controllable promoter, such as the Tet promoter. A first step is the selection of a particular monoclonal antibody with appropriate specificity to the target protein (see below). Then sequences encoding the variable regions of the selected antibody can be cloned into various engineered antibody formats, including, for example, whole antibody, Fab fragments, Fv fragments, single chain Fv fragments (V.sub.H and V.sub.L regions united by a peptide linker) ("ScFv" fragments), diabodies (two associated ScFv fragments with different specificities), and so forth (Hayden et al., 1997, Current Opinion in Immunology 9:210-212). Intracellularly expressed antibodies of the various formats can be targeted into cellular compartments (e.g., the cytoplasm, the nucleus, the mitochondria, etc.) by expressing them as fusions with the various known intracellular leader sequences (Bradbury et al., 1995, Antibody Engineering, vol. 2, Borrebaeck ed., IRL Press, pp 295-351). In particular, the ScFv format appears to be particularly suitable for cytoplasmic targeting.

Detailed Description Text (181):

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L15: Entry 10 of 12 File: USPT Oct 20, 1992

DOCUMENT-IDENTIFIER: US 5156972 A
TITLE: Analyte specific chemical sensor with a ligand and an analogue bound on the sensing surface

Brief Summary Text (10):
5. Bush D. L. and Rechnitz G. A.: "Monoclonal Antibody Biosensor for Antigen Monitoring". Anal. Lett. 20(11) 1781-1790 (1987).

Other Reference Publication (4):
"Monoclonal Antibody Biosensor for Antigen Monitoring"; D. Bush, G. Rechnitz; Analytical Letters, vol. 20, No. 11, pp. 1781-1790 (1987).

For preparation of monoclonal antibodies directed towards a target protein, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used. Such techniques include, but are not restricted to, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256: 495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4: 72), and the EBV hybridoma technique to produce human monoclonal antibodies (Cole et al, 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing recent technology (PCT/US90/02545). According to the invention, human antibodies may be used and can be obtained by using human hybridomas (Cote et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80: 2026-2030), or by transforming human B cells with EBV virus in vitro (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In fact, according to the invention, techniques developed for the production of"chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81: 6851-6855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314: 452-454) by splicing the genes from a mouse antibody molecule specific for the target protein together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention.

Detailed Description Text (185):
In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, e.g., ELISA (enzyme-linked immunosorbent assay). To select antibodies specific to a target protein, one may assay generated hybridomas or a phage display antibody library for an antibody that binds to the target protein.

Other Reference Publication (1):
Eisen, et al., 1998, "Cluster Analysis and Display of Genome-Wide Expression Patterns," Proc. Natl. Acad. Sci., USA, 95: 14863-14868.

Other Reference Publication (10):
DeRisi et al., 1996, "Use of a cDNA microarray to analyze gene expression patterns in human cancer," Nature Genetics 14:457-460.

Other Reference Publication (16):
Schena et al., 1995, "Quantitative monitoring of gene expression patterns with a complementary DNA micro-array," Science 270: 467-470.